

A CONTRIBUTION TO THE BIOLOGY OF
SIMULIUM (DIPTERA)

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INTRODUCTION

IN THE earlier attempts to study *Simulium* larvae in the laboratory, it was claimed that in standing water they would die within a very few hours. Since death occurred too quickly to be due to food reduction, the cause was supposed to be a deficiency of dissolved oxygen. This phenomenon, together with the natural occurrence of the larvae in swiftly running water only, was interpreted as indicating that their oxygen requirement was very high. In fact, it has long been assumed and frequently stated that the restriction to running water is due wholly to so high an oxygen demand that only the much-agitated water of a swift current would provide suitable conditions for these larvae. Though this explanation seemed plausible, no experimental study has ever been made to test it. This paper is the result of an attempt to study the influence of the factor or factors involved in the running-water habitat and to ascertain whether or not oxygen is the sole determining agent, as heretofore supposed. Contributions have likewise been made to the knowledge of the general biology and life-history of these black fly larvae and are presented in Part I.

This investigation was carried out mainly at the University of Michigan Biological Station. All streams in that region were examined, but intensive study and the experimental work were done in Carp Creek, Maple River and Bessey Creek. Some of the laboratory experiments were performed at Ann Arbor with material collected from the Huron River.

* Contribution from the Zoölogical Laboratory, University of Michigan, and the University of Michigan Biological Station.

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PART I. GENERAL BIOLOGY

EGGS

Description

The egg of *Simulium* is generally described as elongate-oval (Malloch, 1914, p. 3) or as triangular with rounded angles (Pomeroy, 1916, p. 11), but examination of the eggs of *S. vittatum* Zett. and *S. venustum* Say showed these descriptions to be based upon two different views of the same egg. One side is somewhat flattened, but the opposite one is humped, the highest point being a little nearer one end. The shape is triangular when so viewed, but if the egg lies on the flattened side and is viewed from above, it is rather oval with both ends broadly rounded. Measurements of the egg of *S. vittatum* are: length, 0.25 mm.; breadth, 0.15 mm.; height, 0.14 mm. Those of *S. venustum* are: length, 0.23 mm.; breadth, 0.14 mm.; height, 0.13 mm. Eggs of both species have tough shells and are attached to the support by means of a transparent sticky substance, which remains elastic in alcohol and can easily be taken off the egg.

The arrangement of the eggs in the mass differs in the two species studied. Eggs of *S. venustum* are, as generally described, placed irregularly in a compact mass (Johannsen, 1903, p. 344; *et al.*), which may be several layers thick. Those of *S. vittatum* are deposited in a string which curves or twists over itself and in which the eggs are laid in a single row, mostly side by side, but sometimes end to end. When oviposition is heavy and the egg-

mass becomes extensive and several-layered, the string arrangement may not be apparent, yet the mass still does not appear compact.

Location

Eggs of *S. venustum* and *S. vittatum* are deposited on leaves of aquatic plants, stones, logs and other objects in the stream. They are usually at or near the water-level. Occasionally some are found above water on vertical objects or on the upper surface of a log or a stone. Garman (1912, p. 20) recorded this unusual occurrence in a general statement: "The eggs were found placed on rocks on the banks a few inches above the surface of water." From observations made in Bessey Creek and Maple River, this position may be due to one or both of two causes operating subsequent to oviposition: (1) fall in level of the stream, and (2) growth of the supporting plant. Stones in Bessey Creek had egg-masses on the upper surfaces just at water-level, but a few days later the same stones were half out of water, with the eggs drying or kept moist by the wavelets. As for eggs laid at the water surface on a vertical emergent plant, it is obvious that growth of the basal regions of the plant would carry them out of water. Such an egg-mass generally had the appearance of a series of bands of different colors, the topmost one being the darkest and the lower ones grading down to lighter shades. This indicates that the eggs at the various levels were of different ages, with the oldest ones highest and those newly laid nearest to the water. Eggs deposited at water-level on different days were carried successively out of water as the plant grew taller. This observation and the fact that oviposition on land has never been recorded indicate that the eggs which Garman found were not deposited on the bank, but were probably left stranded on a plant because of a fall in level of the river.

Development and hatching

At oviposition eggs are creamy white, then gradually change to light tan, brownish tan, brown and finally blackish at hatching. All which develop normally have a shiny appearance; occasionally

some fail to develop and remain dull whitish. As the general color becomes medium tan, the pointed end of the egg appears darker and the head of the embryo larva can be seen. A day or two before hatching, eye-spots become visible. The egg-burster is indicated by a dark spot in the middle of the head, as described by Puri (1925, p. 329).

The emergence slit in the egg-shell invariably occurs at the broad end. After the head pushes out of the opening, the proleg is applied to any adjacent object, securing temporary anchorage, and the rest of the body soon follows. The general shape of this larva is cylindrical, with the head comparatively large and the abdomen short. The larva is immediately active and moves about in a characteristic manner. After a few hours the body assumes the normal form of the older larvae.

Eggs develop normally when kept in still water or in a damp bottle in the laboratory. In the field, eggs have been found to develop in the absence of definite current or in air above water-level. For instance, in the calm protected conditions of Maple Cove, Douglas Lake, eggs and patches of egg-shells often occur on the emergent water plants 20 to 70 feet from the shore. As already mentioned, egg-masses may be left out of water owing to the fall of river level or growth of the supporting plant. All that were kept moist by wavelets and capillary action develop normally. Possibly this explains the statement by Bishopp (1912, p. 384): "Eggs are placed on various objects . . . just above the water level. They hatch . . . and the young larvae enter the water." Barnard (1880, p. 191) made a somewhat similar observation, and stated that the small larvae issued from eggs on the sloping rocks a little above water, and that "from these the young were passing downward into the water to begin an aquatic life."

Incubation period

In natural habitat. — The incubation period for *S. vittatum* was 4-5 days in the latter part of June and through July when the temperature of the water was about 20°-22° C. In order to secure eggs of known age, fresh cat-tail leaves were bent to float at the water surface. Oviposition usually took place in the late after-

noon, and on the next day the leaves bearing the fresh eggs were tied to a stake to keep them well submerged and thus prevent further oviposition. By observation of them until hatching their definite incubation period in the natural habitat was obtained. Pomeroy (1916, p. 12) recorded the minimum period of 7 days and the maximum of 12 days in South Carolina from June to October, but did not state whether it was observed in the natural habitat or under laboratory conditions.

In standing water. — The incubation period in standing water was found to vary greatly, viz., from 5 to 55 days. Several experiments confirmed this statement. Hence the time required in standing water was found to be much longer than in the natural habitat. The incubation periods of the various eggs in the same mass differed; those in the top layer generally hatched earlier than those beneath, although the lower layers had been laid first.

Resistance to desiccation

Edwards (1920, p. 240) suggested that probably *S. latipes* Meig. passes the dry season, when the streamlets are dry for the greater part of the summer, in the egg stage, and Cameron (1922, p. 15) from circumstantial evidence proposed that *S. simile* Mall. probably overwinters in the egg stage. The writer subjected *S. vittatum* eggs to various periods of desiccation in the laboratory and then submerged them in water. A very small number hatched from the egg-mass that had been exposed to the air for 10–14 hours, but not a single hatching occurred from the eggs that had been out of water from 18 hours to 5 days. Evidently *S. vittatum* eggs cannot resist desiccation. Field observations support this conclusion. When, for any reason, eggs were exposed above water, only those that were kept moist hatched. Others beyond the reach of wavelets appeared dry and shrunken, and failed to hatch when returned to water.

LARVAE

Duration of larval period

The larval period of *S. vittatum* was studied in Maple River during the month of July when the variation of the water tempera-

ture was 18°–26° C. (mostly 20°–23° C.). Fresh cat-tail leaves were bent down so that they were slightly submerged and parallel to the water surface. Deposition of eggs and the hatching were recorded, and examination of the larvae was made every second day. Growth was found to be very rapid and the larval stage, for the majority of each brood, lasted only 13–17 days.

Emery (1913, p. 331) found the larval period of *S. vittatum* to be about two months and three weeks. This result was not based on observation of the larvae, but was merely an estimate from the number of broods. This method seems very faulty because the adults appear throughout the summer and it is inaccurate to distinguish the different broods of larvae merely by the relative abundance or scarcity of the flies on the wing. Puri (1925, p. 300) obtained the larval duration of two British species by rearing them in the laboratory. This is a very accurate method for determining the length of the period in aquaria, but it does not necessarily follow that the period is the same in the natural habitat.

In Carp Creek, where *S. venustum* was the predominating species, the larval period was much longer. This was probably due to two factors, namely, the low temperature of the water (10°–14° C. in the summer) and the scarcity of plankton organisms. Observations on this point were made in connection with the trough experiments described in the experimental section. The colonies transplanted into the experimental current troughs usually consisted of larvae of different ages, and they pupated throughout the whole observation period of seven and one-half weeks. At the end of this period there were still some larvae left. These troughs were examined every other day and the larvae were closely observed, so that it is certain that they were all of the original transplanted population. Since the early history of the larvae was not known, the definite larval period could not be determined in this way, yet from the record it is clear that it could be longer than seven and one-half weeks in the summer months. It should be added that, although the observations were made under experimental conditions, the water was from the natural habitat and the current was of various velocities. McBride (1870, p. 227)

recorded the larval period of *S. venustum* to be about four weeks during the warm season, but did not give the temperature of the water.

Habits

Attachment. — The larva is attached by means of the posterior disk, which is provided with rows of hooks arranged in a circle. That this mechanism is not a sucker, as was formerly supposed, was shown by Tonnoir (1923, p. 164). Attachment to the support is accomplished by means of a glutinous material which issues from the mouth and to which the disk is brought in the looping movement.

In experiments on current rates, it was found that the larvae were still well established in a velocity of 6 feet per second. This shows that the attachment must be very firm to resist the pull of such high velocity. Occasionally, dead larvae were found attached to the support, showing that this adherence continued to be effective even after death.

The angle of the body with the support diminishes as the current rate increases. In a moderate current the larva stands semierect, but with increased velocity the angle becomes less and less until the body lies almost prone upon the support. This is very apparent on the more or less vertical surfaces of logs and boulders, on which the larvae are established in waterfalls.

Locomotion. — The looping movement of the larva was described in detail by Tonnoir (1923, p. 104) and by Puri (1925, p. 297). Observations of the writer agree with those of Puri. Besides the regular mode of locomotion, the larva is able to make some progress by means of only the mouth-parts and the proleg, with the abdomen remaining straight and unattached. The process of climbing on a silk thread was often observed. When the larva was on a strand of several silk threads, it either climbed in the regular fashion with the proleg and mouth-parts, or applied its posterior disk to the strand and proceeded with a looping movement.

Food

Being sedentary and provided with a mechanism for straining small particles from the water, the larva does not seem to be selective in feeding, but takes whatever the fans can catch as the current passes through them. Examination of the stomach contents confirmed this general conclusion. Larvae from Maple River contained mostly diatoms, which were very numerous in the summer plankton population of the lake water, but those from Carp Creek had few diatoms and much material that could not be identified, such as bits of plant tissue and other debris.

In the standing water cultures, a few larvae were observed feeding on the deteriorating bodies of dead larvae. Occasionally larvae used their mouth-parts to scrape materials from the substratum. This behavior may have been caused by the unfavorable conditions in the standing water.

Parasites and enemies

Larvae of *S. hirtipes* Fries from Huron River, near Ann Arbor, were parasitized with a nematode, probably *Mermis*, which Strickland (1911, p. 302) found in the same species in Massachusetts. Larvae of *S. vittatum* from Maple River were occasionally infested with a protozoan parasite. Examination of the fresh material showed that the spores and sporoblasts correspond closely with the figures given by Strickland (1913, p. 97), and the appearance of the parasitized larvae also agreed with his description.

In an aquarium containing *S. jenningsi* from Huron River, a *Chironomus* larva was found to attack the *Simulium* larvae. It seized the side of the living victim with its mandibles and began feeding on the soft tissue. It was seen to kill several larvae, but this might be caused by the absence of other food; it does not prove that *Chironomus* is a natural enemy of *Simulium*.

PUPAE

Duration of pupal stage

The duration of the pupal stage has been reported differently by various authors. The longest period given was three weeks

for *S. venustum* Say (McBride, 1870, p. 366) and *S. pictipes* Hagen (Coquillett, 1898, p. 67). But both were mere estimates of the interval between the first appearance of pupae in the breeding place and that of the adults on the wing. Results of direct observation were given by the following investigators: Newstead (1907, p. 40), 2-6 days for *S. ovatum* Mg.; Pomeroy (1916, p. 25), minimum period of $3\frac{1}{2}$ days and maximum 9 days for *S. venustum* Say; Cameron (1922, p. 15), 5-7 days for *S. simile* Mall.; and Puri (1925, p. 302), about 5 days in the summer and longer periods at lower temperatures for *S. nolleri* Fried. Though these workers indicated the temperature conditions, they failed to state whether their observations were made in the natural habitat, in breeding aquaria, or in the damp chamber recommended by Newstead for rearing adults from pupae.

A careful study of the duration of individual pupal stages was made in connection with a trough experiment. Although carried out in the aquarium building, it was in fact comparable to normal conditions, because the stream in the trough was of unmodified lake water and had a velocity of about that of the natural habitat. The V-shaped wooden trough, 54 inches long, was set up with an incline to secure the required velocity. Water was supplied from the large reservoir tank, which was kept filled with fresh lake water by a power pump. Larvae of *S. vittatum* from Maple River were transferred into the trough. They became well established on the sides and gradually pupated. Examination was made three times a day and later twice daily after the majority had pupated and emerged. At each examination all the newly transformed pupae were sketched on the board above the water line and each was assigned a number; later the emergence was recorded according to the numerals. Thus the duration of the individual pupal stage was obtained. The temperature of the water for the first ten days varied between 18° and 21.6° C. After this the average temperature of the water fell $1\frac{1}{2}$ degrees, but only 7 per cent of the pupae were left in the trough. Altogether 101 larvae pupated. Ninety-one completed development; of the other 10, 7 died, 1 was removed for examination, 1 was lost, and 1 had not emerged when the experiment was discontinued. The curve

plotted from the resulting data shows a prominent mode at $4\frac{1}{4}$ days, with a minimum period of $3\frac{1}{4}$ days and a maximum of $5\frac{1}{4}$ days. The total variation is 2 days, but 66 per cent have a variation of only one-half day, their periods being between 4 and $4\frac{1}{2}$ days. Of the rest, 16 per cent fall between $3\frac{1}{4}$ and $3\frac{3}{4}$ days and 17 per cent between $4\frac{3}{4}$ and $5\frac{1}{4}$ days (with 5, 9, 10 pupae).

Cocoon-spinning and pupation

Spinning of the cocoon.— Before the larva actually starts spinning, it performs a series of preliminary motions: it bends back and applies the mouth-parts to a certain point on the support near its posterior end, then suddenly pulls its head away with some force. It repeats this performance many times, testing various areas around itself on the supporting object. In one instance this was observed to last for more than one minute. The purpose of this behavior appears to be the cleaning of the ground of débris, or the testing of the firmness of the substratum.

The process of cocoon-spinning was fully described by Tonnoir (1923, pp. 165–169) for *S. tillyardi*, an Australian species, whose cocoon is almost exactly circular. Puri (1925, p. 301) gave a brief account of three British species whose cocoons are of the wall-pocket type. The writer has studied the spinning of *S. venustum* Say, but found it to differ from Puri's description in that the larva never changes its attachment throughout the whole process. This point agrees with Tonnoir, although the *S. venustum* cocoon is not circular, but like a wall-pocket. Firmly anchored by means of its posterior disk, the larva bends back and places a loose network of silk on the supporting object just posteriorad to its point of attachment. Then it works forward along the side of the body, until the bend is at about the anterior third. After fixing the silk to the support it twists so as to turn the ventral side outward and upward, and produces a strand of silk while describing an arc dorsally over its body to a similar point on the opposite side. Repetition of this movement results in the formation of the rim of the future cocoon. Since the larva does not change the point of attachment, the two ends of this arc are in fact the extreme points, in front, to which the larva can reach

when bent at its anterior third. The limit to which it can stretch behind naturally becomes the apex of the case. With these three points fixed, the larva spins more threads along the sides and dorsally over its body, and the cocoon soon takes definite shape. Then it spends some time working over the closed end of the cocoon. It takes from 50 to 60 minutes to complete the whole process. The network which is put down at the beginning becomes the floor of the posterior part of the cocoon, the anterior margin of which marks the place where the larva is attached while spinning, and this explains why the front of the cocoon has no bottom.

Process of pupation. — The pupation process was found to be quite similar to that described by Tonnoir (1923, p. 169). Doubled into the form of a U, back to back, the larva rotates for from 10 to 15 minutes. A slit occurs along the lateral and the posterior borders of the fronto-clypeus and continues ventrad between the head and thorax. The gill filaments emerge, the head capsule shell comes off the front, and the rest of the larval skin is cast from the posterior end as the pupa withdraws from it and straightens out inside the cocoon. For a few minutes slight sidewise wavy movements continue in the posterior region. By the time the pupa comes to rest mouth-parts and wing pads are already traceable.

In standing water. — Pupation in standing water was observed many times. Numerous individuals fail to come out of the larval skin and die with only the thoracic region enlarged and gill filaments bursting out at the sides. Some may complete pupation, but with no cocoon or only a shapeless mass of silk threads. Others build their cases and pupate in the normal manner. The only difference between such a pupa and one formed in running water is that the gill filaments in the former spread out in all directions, while those in the latter lie rather straight in the direction of the current.

Movements of pupa

Turning within cocoon. — Inside the cocoon the pupa is able to move or turn rather freely. Pupae have frequently been seen on the side or with the ventral surface upward. The most active

motion observed was when a pupa was disturbed by a neighboring full-grown larva clearing the ground for cocoon-building. When the larva touched the anterior end or the gill filaments of the pupa, the latter performed the avoiding reaction with its anterior region.

Desertion of cocoon. — McBride (1870, p. 366) observed that the pupa of *S. venustum* loosened itself from the case a day or two previous to emerging. Puri (1925, p. 301) claimed this to be wrongly described, because "once a pupa has entered the cocoon, it is fixed to the latter and never leaves it of its own accord." Though this is the normal course, the writer has, however, observed pupae, some of which transformed later, leaving their cocoons under distinctly unfavorable conditions. Emery (1913, p. 336) recorded a similar observation, but did not give details. However, the statement that they "faced in the opposite direction alongside of the cases" indicates that they could not be under normal conditions.

Enemies and parasites

A Chironomus larva was observed attacking pupae in an aquarium. It penetrated the side of the abdomen and fed on the soft tissue inside, leaving the pupal skin clean and intact. In the field no such empty pupal skins have been found, but dead pupae were occasionally encountered, most of which had a puncture in the thoracic or abdominal region while the internal tissues were in various states of deterioration. It has not been ascertained what enemy attacked them.

Dissection of pupae yielded no evidence of parasites. The only record of parasites in Simulium pupae was given by Enderlein (1921, p. 198), who obtained two braconids belonging to two subfamilies from a group of 15-20 pupae. He stated that this was the only time he obtained parasites, although he had reared thousands of Simulium flies from pupae.

Emergence

Process of emergence. — The process of emergence was studied in the natural habitat and in the aquarium. The pupa appears

silvery, owing to the presence of gas underneath the pupal integument. The relation between gas accumulation and the splitting of the exuviae is not known, although it was found that pupae may manifest the silvery appearance for more than one hour before the splitting occurs. As soon as the split along the mid-dorsal thoracic region takes place the adult thorax is exposed under a layer of silvery gas. Then the slit extends between the head and thorax and along the lateral margins of the front piece covering the vertex of the adult fly. Gradually more of the thorax comes out, carrying with it the head, which is still bent, facing downward, and does not assume the normal position until it is all free from the pupal skin. At the same time an undulating movement goes on in the abdomen. After the head and most of the thorax are free, the fly comes up faster — the direction is more vertical than forward — until only half, or less, of the abdomen is still inside the pupal skin. It takes the fly 2–4 minutes to come out so far; then it rests for about one minute. Throughout the whole period the fly has not struggled any in emerging from the skin; during the interval of rest it may remain perfectly quiet, or the front end may wave slightly and slowly sidewise. Then it begins to come up again; as soon as the front legs are free and wriggling, it is immediately carried upward in the gas envelope, and on reaching the surface flies away at once. Cameron (1922, p. 16), Coquillett (1898, p. 67) and Pomeroy (1916, p. 26) also found the adult to take flight almost immediately upon coming up to the surface of the water. If, according to certain other workers (Emery, 1913, p. 337; McBride, 1870, p. 356), it stays on the surface for the wings to dry, the current must be moderate; otherwise it would be carried downstream and possibly suffer accident.

Emery (1913, p. 337) gives an account of emergence of a pupa on a stone when held up in the air. This should not be taken for the normal process under water, for the buoyancy of the gas coming off the fly renders the condition quite different. According to the observation of the writer, the fly never exerts any "pulling"; and after the thorax is out, it is only by having part of the abdomen and tips of the legs still inside the pupal skin that *rise* to the

surface is delayed. It has not been observed to pull out its legs until ready to come up with the gas envelope.

Emergence in air. — Newstead (1907, p. 40) reared adults by keeping pupae in a damp chamber. Flies are thus able to emerge in air as well as in water. Pomeroy (1916, p. 25) noted the fall in river level, leaving the pupae exposed to air, and added that they would emerge even after having been 24 hours out of water if the adult is sufficiently developed. Recently Pulikovsky (1927) studied the respiratory adaptation of *Simulium* pupae and called the pupal gills "amphibian" in nature, because they function as respiratory organs both in water and in air. His work was on a species inhabiting mountain streams that dry up easily. From one of his experiments (p. 418) he concluded: "Die Puppe verlangt am Anfang ihrer Entwicklung notwendigerweise die Gegenwart von Wasser; weiterhin fällt diese Notwendigkeit fort (trocken gewordene Puppen sind entwicklungsfähig)." It seems to the writer that there are other factors to be considered besides the degree of development itself. Since pupae are able to develop normally when removed to a damp chamber even immediately after pupation, it proves that submergence is not indispensable for early development. The really significant factors, it seems to the writer, are (1) the humidity of the surrounding air, and (2) the degree of chitinization of the pupal skin. The newly transformed pupa is pale in color, and as chitin is deposited it gradually changes to tan, light brown, and finally black when the fly inside is fully developed and shows through the pupal skin. If the surrounding air is humid, development is not affected, even when the pupa is not protected by a well-chitinized integument. But when such a pupa is subjected continuously to dry air, it may be that the tissue inside loses water through evaporation and becomes too dry for normal development. When the pupa is advanced in development and protected not only by its own chitinized integument but also by that of the future fly, the dry air will not do any harm.

The writer has studied pupae above the water-level in natural habitats and pupae that were kept dry, experimentally, for various periods. On vigorously growing cat-tail leaves in Maple River, pupae were occasionally found from 1 to 10½ inches above water.

These were in different stages of development and the cocoons were almost invariably dry. Of 45 pupae observed, 34 produced imagoes; 4 were lost from the leaves; and 7 died. Four of the dead were very dry and shrunk into the cocoon, and 3 showed the thorax of the fly partly projecting through the emergence slit and appearing dry also. Excluding the 4 that were lost, this record shows a mortality of 17 per cent apparently due to dryness. In the laboratory pupae on their natural support were exposed to the air for different lengths of time and then put into damp chambers. Of 21 pupae so exposed for 24 hours, 17 emerged and 4 dried up. Of 6 others exposed for 34 hours, 4 black and brown pupae emerged and the 2 tan ones became dry. Of 6 additional ones exposed 54 hours, only 1 emerged and that within a day, showing that it was advanced in development when subjected to desiccation. Ten pupae remained in the dry air, to which they had been exposed for 54 hours. Of these not a single emergence occurred. From these results it is evident that there is a desiccation limit beyond which the pupae are unable to complete development.

Pupae in standing water

The writer has found that most of the pupae kept in standing water failed to complete development. This was true of pupae collected from the field and of those that pupated in standing water.

IMAGO

Duration of the adult stage

Since it was impossible to make direct observation on the duration of the adult stage in nature, flies have been studied only in captivity. Friederichs (1921, p. 83) succeeded in keeping the flies alive longer than other investigators did. He reared *S. maculatum* Meig. on sugar solution in a very large cage and had 2 females that lived 23 days; the majority died within 14 days. Blacklock (1926 a, p. 12) experimented with *S. damnosum*, and only 1 out of 268 lived for 10 days, the majority dying within 4 days.

The writer, working on *S. vittatum* and *S. jenningsi* Mall.,

used sugar solution, soaked raisins or water alone. For 52 flies supplied only with water, the longest period was 6 days for 1 female. The majority died within 3 days; 5 males and 5 females lived for 5 days. When sugar solution or soaked raisins were provided as food, the period was greatly lengthened. Both males and females were seen to feed on it. The longest period obtained was 18 days for 2 males and 3 females of *S. vittatum* and for 2 females of *S. jenningsi*. The majority died within 8 days, but there were individuals that had a period varying from 9 to 17 days. These experiments included altogether 223 flies. In general, the female outlived the male in the different lots. Only in one lot fed with sugar solution did both male and female flies live to the end of 18 days.

Swarm

Cameron (1922, p. 16) described the various kinds of swarms, which consist of females, males and mixtures of both. Enderlein (1921, p. 198) mentioned a swarm of males as the only one seen during ten years' collecting. Near the Biological Station small swarms of females are very common in the late afternoon, especially after a warm, clear day. But swarms of males are seldom seen unless one looks particularly for them. They form higher up in the air, at least about 10 feet from the ground. Most flies captured from such a swarm are males, but from the behavior of the flies it appears to be a mating swarm. The flies hover steadily for a while and then suddenly dart for short distances. On calm evenings such swarms often form in open exposed places; but when there is wind, they are found in more sheltered positions, such as small clearings among the woods. One evening while collecting flies among the aspens near the Biological Station, the writer saw a small swarm of females over a sweater thrown on a stump, with many individuals searching and burrowing among the woollen fibers.

Sex ratio

Collectors of *Simulium* very rarely capture male flies by the net, and the writer had the same experience in the Douglas Lake

region. The small number of males in the field led to a study of the sex ratio in the reared specimens. The following figures show in every case an approach to the 1:1 ratio: *S. vittatum* Zett., 706:707; *S. jenningsi* Mall., 135:145; and *S. venustum* Say, 35:43.

Oviposition

Place. — Oviposition has been observed by different investigators and found to be of two types: (1) below the surface of water, and (2) at the water-level. Britten (1915, p. 170) and Edwards (1920, p. 237) described the oviposition of two British species at a depth of from a few inches to one foot under water, and Comstock (1925, p. 823) observed that *S. pictipes* Hagen deposited the eggs on the rocks by darting into the thin sheet of water and out again. Records (Webster, 1887, p. 458; Needham, 1901, p. 408; Knab, 1916, p. 281) of the process in other American species show that they all belong to the second type. The observation of the writer on *S. vittatum* agrees with those of Emery (1913, p. 330) and Pomeroy (1916, p. 8) on the same species, that is, eggs are deposited at or near the water-level. Eggs of *S. venustum* have been found on grass blades also near the surface of the water.

The fly, in search for a suitable oviposition place, hovers over the water and alights on some object just above the water-level. Generally it selects a place with a thin film of water, or where its front legs can rest on dry ground while the tip of the abdomen reaches the water's edge. In Maple River the many long cat-tail leaves, bent down and floating in the stream, present a very favorable condition for egg-laying. Although the body of the fly is normally above water, it remains undisturbed if the swaying of the leaf should carry it under water for a moment. It is enveloped within an air bubble and often continues laying eggs. Flies have been submerged by holding the leaf under water for 1-5 minutes. Some of them stayed in the same position; others crawled about, and, letting go their hold, rose up to the surface within the air bubble; all were dry upon emergence from the water.

Oviposition occurs also outside the running stream. In the

shallows of the protected Maple Cove eggs are often found on the stems and bent-down leaves of small emergent plants. These may be quite near the shore-line or farther out in the lake up to a distance of 70-80 feet, and they are from 50 to 120 feet from the actual breeding place.

Time. — In northern Michigan oviposition occurs from about 4 o'clock P.M. to dusk, with the greatest activity from about 5 to 8 P.M. After a calm, warm day the flies are most active, and very numerous. In Maple River, cat-tail leaves, bent down to floating position in water, are literally covered with egg-masses, several layers deep, after one evening's activity. Sometimes the masses even extend beyond the edge of the leaf. Occasionally fresh egg patches were found in the early morning, but no oviposition was observed, nor were newly laid eggs seen between 9 A.M. and 4 P.M.

Throughout the season, from the middle of June to the middle of August, eggs were found in Maple River, but the heavy oviposition lasted about four weeks. In 1926 it was already heavy when the first record was taken on June 15; the numbers decreased after July 10. In 1927 the period of extensive laying began toward the end of June and lasted longer than during the previous season. On days of great activity hundreds of flies were hovering close to the water and producing a low buzzing sound. Often 4-6 flies were seen ovipositing at the same spot; a section of a cat-tail leaf one foot long might have as many as 50-60 flies depositing eggs at the same time. Usually a fly does not deposit all the eggs at one place, but often crawls to some other spot or flies away and then alights again to continue the process. Flies have been observed to continue egg-laying for 1-3½ minutes; most of them deposited the eggs in a string along the water edge.

Number of eggs. — Malloch (1914, p. 8) pointed out that the large number of 5,000-10,000 eggs from one female recorded by Horvath was undoubtedly an exaggeration, being probably an estimate of eggs in one mass deposited by a number of flies. Friederichs (1921, p. 86) counted 500-1,000 eggs in the small egg patches that appeared to be deposited by one female. Riley (1886, p. 504) gave 500 as the number of eggs found in the ovaries of the American species. In *S. vittatum* the writer counted 300-320 well-

developed eggs in the ovaries of reared specimens which had not oviposited.

Mortality. — Many females, dead or struggling to escape, were found stuck to the gelatinous matrix of the egg-mass. On one occasion over 30 flies were counted on a section of a leaf about $1\frac{1}{2}$ feet long. Pomeroy (1916, p. 9) observed a similar condition on the egg-mass on bridge piles.

Many of these flies were removed and dissected for examination of the ovaries. Some had finished egg-laying; others contained eggs varying in number, the highest being 250 in one fly. This indicates that they became stuck by accident during oviposition. The newly laid eggs are very sticky, but older eggs are no longer so, since the flies can crawl freely over the tan-colored ones and continue to deposit new layers of eggs without becoming trapped.

In captivity. — All attempts to induce oviposition in captivity failed. Cages placed in the open under trees were provided with dishes of water containing aquatic plants and pebbles. Thick sugar solutions or soaked raisins were supplied as food. Both reared specimens (*S. vittatum*) and those caught with the net (*S. venustum*) were used. When flies did not oviposit under such conditions, a cage was placed in the edge of the lake so that water in motion would be provided. This is identical with the condition in Maple Cove, so far as water and objects for attachment are concerned, yet no oviposition occurred. As concerns *S. vittatum*, this failure was not due to a lack of proper food for the development of the ovaries, because the flies, when dissected, contained mature eggs. Discussion of *S. venustum* in this connection appears later.

Oviposition was accidentally secured from two reared *S. vittatum* flies. One female was isolated after emergence in a 6-dram vial. Water was supplied on a small piece of cheese cloth, but no food was given. On the fifth day the fly died, but had laid eggs on the wet cloth. These unfertilized eggs failed to hatch. From dissection it was found that all the 152 eggs were from one ovary; the other was not well-developed and the eggs were less than half-sized. In the other instance about 80 eggs were found on the moist wall of a half-pint jar in which both males and females had

been kept. No mating has ever been observed in captivity, and these eggs did not hatch; probably they also were unfertilized.

Food

The food of *Simulium* flies, except the blood-sucking ones, is not definitely known. In captivity, individuals of both sexes of *S. venustum* and *S. vittatum* take sugar solution readily and live very well on soaked raisins. They also feed upon fresh honey-suckle leaves and take water from droplets on them. Both species are recorded as blood-sucking, but *S. vittatum* was not found biting, nor did it seem to be associated with *S. venustum*, which is very annoying in the Douglas Lake region.

The fact that both males and females can live 5-6 days without any food proves that food is not an absolute necessity, at least for that length of time after emergence. The presence of fat-bodies in both sexes may serve to explain, at least in part, why they do not depend upon the taking in of nutritious material from the outside, even when they are active on the wing. The fat-bodies in newly emerged females of *S. vittatum* are very large, but as the eggs develop they gradually decrease in size. Two flies kept on water alone oviposited in captivity. This significant evidence indicates that the flies not only lived, but also completed the egg development and oviposition without taking any food. Water is necessary, for if they are not supplied with it they die within 2 or 3 days. Perhaps this supports the general supposition that the flies which do not suck blood live on plant juices.

Blood sucking and development of ovary

The dependence of ovary development on a blood meal was studied by Pomeroy in *S. venustum* Say (1916, pp. 30-34) and confirmed by Cameron in *S. simile* Mall. (1922, pp. 19-20). The writer also found that, without the engorgement of blood, the *S. venustum* fly, either reared or captured, never contained ovaries with developed eggs, although eighteen of the flies dissected had lived for 9 days. But *S. vittatum*, although known as a blood-sucking species, does not attack man in the Douglas Lake region, and so presents an interesting condition for study.

As mentioned in the section on oviposition, two specimens of *S. vittatum* deposited eggs in captivity without taking any food at all. Other reared specimens kept on sugar solution or water alone have been dissected, ranging from those just emerged to those 5 days old. Ovaries of newly emerged flies contained small round ova, corresponding to what Pomeroy called stage 2. After 1 day, the eggs were about one half the size of mature ones and some began to assume the oval shape. After 2 days, they were about three fourths mature, although one of the eight flies dissected contained fully developed eggs; after 3 days, the eggs became mature and had the typical triangular shape. They were very sticky, adhering to the needle and scalpel during dissection. When the flies lived for more than 4-5 days the eggs showed no further growth. From this study it is evident that in *S. vittatum* the development of the ova is not dependent on a blood meal or any other food, and that the fat-bodies, probably present, supply the necessary material for growth (see section on food).

The presence of fat-bodies is, however, not an indication that the female does not need food for ovary development. Both *S. venustum* and *S. jenningsi* Mall. have them, though proportionately less than those in *S. vittatum*, but when kept under the same conditions as *S. vittatum* their ova *do not develop at all*. This tends to show that the blood-sucking forms require a definite food in order to produce progeny, yet *S. vittatum* appears to be an exception. As mentioned at the beginning, *S. vittatum* is not a pest to man in the Douglas Lake region. But if it should be found to attack animals, it would prove to be a form that could complete its ovary development with or without a blood meal. The condition of the alimentary tract of the flies captured while ovipositing gave no indication that they had taken blood. Blacklock (1926 b, p. 210) found small amounts of altered blood up to 4 days after breeding. Therefore, if *S. vittatum* does not suck blood at all in the Douglas Lake region, it must be a species which has different food habits in different localities. It is definitely a biting species in Kansas; Hunter (1914, p. 311) used it in the experiments on the attempted transmission of pellagra.

In this connection two other instances may be mentioned.

S. venustum, the well-known pest, is recorded by Needham (1901, p. 408) as "peaceably disposed" in the Adirondacks, and by Sanderson (1910, p. 27) as "causing very little annoyance in the White Mountains." *S. hirtipes* Fries is the species accused by him of biting; Comstock (1925, p. 824) also called it the scourge of the fishermen in the mountains of the northeastern states. Near Ann Arbor *S. hirtipes* occurs in the Huron River. A few reared specimens were kept on sugar solution, and two flies, 4 and 5 days old, contained ovaries with well-developed eggs. Although the number is too small to be significant, distinct differences from the ovaries in *S. venustum* and *S. jenningsi* were shown. If this indicates a food habit different from these two species, then *S. hirtipes* is not a blood-sucking form near Ann Arbor.

Both Pomeroy and Cameron found the simultaneous occurrence of mature and immature eggs in the ovaries of certain *captured* adults, but they had no way of knowing the history of these individuals. It may be of interest, therefore, to note that the ovaries of the two specimens of *S. hirtipes* mentioned above contained ova of two sizes: (1) about 30–50 mature ones, and (2) a large number of the small, round type. Newly emerged females have been examined; their ovaries contained small ova of uniform size. These two flies were fed with sugar solution. One died after 4 days and the other had lived 5 days when dissected. Both had *not* oviposited, so that here the condition is different from that which Pomeroy called "stage 5," that is, a few mature ova left from an earlier oviposition in the ovary with rudimentary eggs.

The writer has found two cases corresponding to "stage 5" among individuals stuck to egg-masses. The condition of these small ova does seem to indicate, as Pomeroy stated, that they may be developed later for another oviposition, but the writer has not obtained enough evidence to support such a supposition.

Parasite

Two nematode parasites were found when preserved adults were dissected. One *S. hirtipes* Fries female contained a large worm 17 mm. long, which filled the abdomen completely. One *S. jenningsi* Mall. female had a small worm about 3 mm. in length

coiled around its ventriculus. Both imagoes were reared from pupae collected from the Huron River, in which locality the *larvae* of these two species were found to be occasionally parasitized by nematodes.

The writer is able to find only one record (Swinton, Edwards and Boylis, 1913, p. 391) in the literature of a nematode in a *Simulium* adult. Swinton found a specimen of *S. ornatum* Mg. on a window-pane; after killing it with a drop of benzine, he suddenly noticed a worm coming out of the abdomen of the mounted specimen. In his paper on the parasites of *Simulium* larvae, Strickland (1911, p. 326) concluded that *Mermis* "inhibits the development of the histoblasts to such an extent that pupation becomes impossible." The two worms found by the writer must have been carried over from the larval stage, because the reared flies were dropped into alcohol soon after emergence and had no time to become infested. Strickland (p. 311) pointed out that in the larvae parasitized with histoblasts of moderate size the worm may have entered the body cavity after the disks were somewhat developed. This possibility may explain the presence of the small worm in *S. jenningsi*. It might thus have succeeded in pupating because of late infestation, but the large parasite in *S. hirtipes* appears to be an exception to the observations of Strickland.

PART II. EXPERIMENTAL SECTION

GENERAL STATEMENT

It has long been known that the exclusive breeding place of *Simulium* is in swiftly running water, as is well attested by the numerous literature records on this point listed by Wilhelmi (1920, p. 133). But after reviewing the literature one is surprised to find only the mere recognition of this fact and no study at all of the factors involved. In spite of the fact that running water is claimed to be essential to the life of the larvae, not one measurement of the actual velocity of the current in the habitat was taken by these investigators. The only record of which the writer is aware is that of Tonnoir (1925, p. 216) in his study on Australian *Simulium*, namely, that the larvae are found "only where the

speed of the current is about one foot per second." Furthermore, with no other evidence than that the larvae usually died within a few hours in standing water, earlier investigators claimed that the oxygen requirement of these larvae is very high and can be satisfied only by the much-agitated water in a swift current.

In order to make a thorough study of this requirement of the larvae for running water, experiments have been carried out to analyze the possible factors concerned and to ascertain the relative influence of each. The experiments fall into three groups: (1) on current rate, (2) on the food factor, and (3) on the oxygen supply. All experiments in the natural habitats were performed in the Douglas Lake region. Laboratory experiments were conducted both at Ann Arbor and at the University of Michigan Biological Station.

EXPERIMENTS ON CURRENT VARIATION

Current rates in natural habitats

Before experiments were planned, a study was made of the current rates in the natural habitats in the Douglas Lake region. The current was measured with a small glass tube bent at right angles and the results were calculated from the following formula: $V = 0.977 \times \sqrt{2gh}$. V = velocity, in terms of feet per second; g = gravity, 32.16 feet per second; and h = height of water column in the upright arm of the tube, when the other arm is set in water against the current. Correction was made in the value of h by subtracting from the actual reading the effect of capillarity.

A careful study of the water velocity in situations where *Simulium* larvae occurred, except in the vertical falls, showed that the current rate varied from 0.56 to 2.75 feet per second, with the majority of the records occurring between 1 and 2 feet per second. The current rate was even greater where the water drops over boulders or log dams in a perpendicular fall, but the glass tube was not usable in this position. Although the highest possible rate in the natural habitat has thus not been specifically determined, the minimum rate, which was of greatest importance in this connection, was critically studied, especially in Bessey Creek.

The upper channel of this stream consists of stretches of shallows with sandy bottom connecting deeper depressions at the concave or outer side of the numerous bends. These connecting slopes are favorable locations for a series of measurements, since they represent a natural current rate gradient and support a population of *Simulium* larvae and pupae. Three typical slopes were selected and four series of measurements were made. The minimum rate, i.e. that below which no *Simulium* larvae occurred, was found to be 0.56 foot per second in two of the series, 0.79 foot per second in another, and 0.97 foot per second in the fourth. In a small side channel, where a group of larvae were established on Potamogeton leaves, the current was also 0.56 foot per second. This minimum rate as determined is for Bessey Creek only, where *S. vittatum* and *S. venustum* are the predominating species; it should not be taken as the minimum rate for all species and in all kinds of streams.

Sediments. — In connection with the study of current, it was shown clearly that, when other factors were equal, the sediment on the supporting objects had a close relation to the presence or absence of the larvae on them. For instance, in the third of the series referred to above, the minimum rate, instead of being 0.56 foot, was 0.79 foot per second, but there was no environmental difference between this and the first two series other than the presence of sediment on objects at points with lower rate. Even at a point with a rate of 0.79 foot per second, larvae were present only on the under side of a leaf the upper surface of which was partially covered with sediment. At a position with the same rate, in the fourth series, similar leaves had a rather thick layer of sediment on both sides and no larvae were found; under these circumstances the minimum rate was 0.97 foot per second. The cause of sedimentation differences under the same velocity in the same stream is unknown, but it appears to be either or both of two things: (1) the time through which sediment has been accumulating, or (2) the relative distance from the shallow swift section upstream.

Transplantation

In studying the current variation it is necessary to secure such conditions that, while the current rate varies, the other factors remain unmodified. Several streams in the Douglas Lake region presented such favorable conditions because within a short distance of places where larvae occur normally the velocity may become much lower while the food material and the chemical nature of the water remain unaltered. This favored the transplantation work which was carried out in Maple River, Carp Creek and Bessey Creek.

In Maple River

Maple River arises in the extreme end of Maple Cove. Quiet lake water is thus gradually converted into unaltered running water. The current becomes about 1.5 feet per second near a cat-tail patch about 20 feet downstream from the lake margin. Here larvae suddenly appear in great numbers, mostly on long cat-tail leaves that are bent and floating in water, but also on Potamogeton and other plants. From this point lakeward (upstream) the current decreases gradually until it disappears in the shallows of the cove. This section with varying rates of current was selected for the transplantation experiments.

Seven stakes were set in a row, with the first nearest to the cat-tail patch and the others successively upstream with the last farthest out in the lake. Cat-tail leaves bearing larvae and pupae were cut off at the leaf base and gently dragged in water to the different stations and tied to the stakes, care being taken not to dislodge the larvae by jerking the leaves too much or lifting them out of water. At the same time fresh cat-tail leaves were tied to each station to provide more objects for attachment. The current rate at each station was measured. In general, there was some decrease in the numbers after the transplantation, but if the majority remained they were recorded as established. Records were taken every second day for 20 days and at longer intervals subsequently. From observations extending over six weeks the following results were obtained:

Station 1. — Current variation, 0.97–1.26 feet per second throughout period of observation. Larvae well established and pupated normally. Station observed for only 16 days, owing to disappearance of stake supporting transplanted leaves.

Stations 2–3. — Stations located near beginning of river. Station 3 three feet upstream from Station 2 and slightly within margin of lake. Current variation 0.79–0.97 foot per second during whole period of observation. Transplanted larvae well established and subsequently pupated. Eggs hatched and resulting larvae thrived. Later in the season, on fall of the lake level, the current rate was nearer 0.79 and larvae decreased in numbers, though some of various sizes present till end of observation period.

Stations 4–5. — Out in lake; 2 feet apart; Station 4 about 10 feet from Station 3. Current 0.58 foot per second; sometimes slightly higher. Transplanted larvae, mostly fifth and sixth instars, established at both stations and pupated. Larvae hatching there disappeared after reaching fourth instar.

Station 6–7. — Station 6 about 15 feet farther in lake from Station 5 and Station 7 about 5 feet beyond Station 6. Current 0.40 foot per second (lowest rate measurable by available apparatus); not detectable later in season. Larvae disappeared from supporting leaves; very few remaining 2 or 4 days after transplantation. Larvae, full-grown at transplantation, pupated and emerged successfully. Eggs, deposited locally or transplanted, hatched, but resulting larvae disappeared within 2 or 3 days.

Results. — When larvae were transplanted from their normal habitats to localities with all conditions the same, except for reduction of the current velocity, they became established only where the current rate was 0.58 foot or more per second; the medium-sized larvae of the fourth instar appeared to require a current higher than 0.58 foot per second.

In Carp Creek

Carp Creek is a swift stream fed by underground water and flows through a deep, wooded valley into Burt Lake. About half a mile from its mouth a beaver dam over 200 feet long and 3–5 feet high produced a waterfall of considerable volume near the

steep east bank. *Simulium* larvae, *S. venustum* mixed with *S. vittatum*, occurred in large numbers in the overflow, but were present in the creek in smaller numbers. The beaver pond above the dam was very extensive; at its beginning, where the swift stream became gradually converted into the quiet water of the pool, there was found a natural graded series of current velocities. This favorable location was selected for transplantation work to find out the minimum rate required.

Convenient objects in the stream were chosen as the stations and the current rate at each was measured. In the first series, four stations had the following rates per second: Stations 1 and 2, 0.97 foot; Station 3, 1.49 feet; Station 4, 1.87 feet. Sticks bearing a good supply of larvae were collected from below the beaver dam and were tied securely at the different stations. When examined the next day, larvae were established at Stations 3 and 4, but had disappeared from Stations 1 and 2. Apparently the minimum rate was between 0.97 and 1.49 feet per second.

A second series of four stations was then selected. Sticks with a good supply of larvae were collected from below the beaver dam and carried over in a long shallow pan containing water. This proved to be a good method. The results from observations of one week were as follows:

Station 5. — Current rate 0.97 foot per second. After 2 days only 2 larvae out of about 20 were found on the stick, which was already fairly well covered with sediment; all had gone after 4 days.

Station 6. — Current rate 0.97 foot per second. After 2 days only 1 larva out of about 50 was left on the stick, which was covered with sediment. After 4 days no larvae were seen.

Station 7. — Current rate was 1.26 feet per second. Larvae well established. After 6 days most of the larvae shifted to the under side of the stick when the upper surface was covered with sediment.

Station 8. — Current rate was 1.26 feet per second. No larvae were present on the next examination, but the stick was much lower down than when it was fixed, indicating that it might have been interfered with in some way.

Station 3 of Series 1. — Current rate was 1.49 feet per second. Larvae were established and still present on the seventh day after transplantation. Sediment was gradually accumulating; larvae shifted to the under side.

Results. — Larvae transplanted from a locality with a rate of 2.75 feet per second became established at a rate of 1.26 feet per second. Records of Stations 5 and 6 tend to indicate that they might establish themselves where the current rate was 0.97 foot per second, if the factor of sediment was eliminated.

In Bessey Creek

Only one series of three transplantations was made; no larvae were established at the new localities, but interesting observations were made on the behavior of the larvae immediately after the transfer from their normal current rate to a weaker one. In transplantation the plant leaf supporting a colony of larvae was cut off and gently dragged in water to the selected spot. Very few larvae were lost through this handling. But soon after the leaf was fastened the larvae became restless; they changed their point of attachment and turned their heads in different directions, as if testing the movement of the water. Very often they shifted to the under side of the leaf, or along the lower edge if the blade was in a vertical position, and were established temporarily. Some larvae actually loosened themselves from attachment and, as they spun the silk thread, were carried slowly downstream. They were able to stop spinning and remain suspended for a while to investigate their environment. Some began climbing back on the silk thread, but very few reached the original position. If the thread became attached to some neighboring object, the larvae crawled upon it. Most of them would start spinning again and were soon carried away downstream. On one occasion approximately one half of the original population were lost from the leaf within 20 minutes. This was a transfer from a habitat with a rate of 1.87 feet per second to a place where the current was 0.56 foot per second. In one transfer from a current rate of 1.26 feet per second to one of 0.40 foot per second the behavior of the larvae was the

same; within 15 minutes most of the large and medium ones had disappeared from the upper surface of the leaf.

DISCUSSION

From the results obtained from transplantation work, there are several points of interest to be considered.

(1) When larvae are transferred to a current weaker than that of their normal habitat, some of them let go their hold within a very short time, as if in search of a more favorable environment. Obviously, this behavior is not due to the food relation, because they could not have suffered, within a few minutes, from any decrease in the amount of food carried to them by the slower current. The important factor of oxygen supply does have immediate effects upon organisms and it seems to explain the response of the larvae on the supposition that the weaker current cannot meet their high oxygen requirement. But when the situation is examined closely, such an explanation cannot be applied. The dissolved oxygen content of the surface water is high, and, furthermore, the water in contact with the body surface and anal gills is changed even with very slow current. Under such conditions it seems difficult to suppose that the larvae are unable to effect adequate exchange of gases. The very fact that not all the larvae left the new environment simultaneously is a proof that the cause of their restlessness is not suffocation. It will be remembered that in the study on the current rates in the natural habitat in Bessey Creek a small number of larvae were found living normally in a current of 0.56 foot per second. It is evident from these considerations that the immediate desertion of the new locality is not a vital necessity. This behavior appears, therefore, to be a mere response to the change of pressure. It is obvious that, with the slower current, the pressure of the running water against the body surface is diminished and that this difference is the first thing the larvae experience in the new environment. It seems reasonable to suppose that, after finding themselves out of their normal surroundings, they would become restless. It may be, therefore, merely a response to the immediate stimulus without involving relation to the other factors.

(2) A small number of larvae remained at stations in very weak currents for a few days, but finally disappeared. This was observed in transplantation in both Maple River and Carp Creek. Although they did not become established, the very fact that they lived for 3 or 4 days where the current rate was only one third of that of their normal habitat was significant, and showed that they secured oxygen from the slow-moving water. On the other hand, the final disappearance of the larvae indicated that there were factors other than oxygen supply involved in their requirement for swift-running streams. In Carp Creek, it appeared to be a matter of sedimentation, for the larvae disappeared when their supporting object was covered with sediment. In Maple River, there was no apparent cause for their disappearance, but there was the problem of insufficient food. If only one third of the normal volume of water passed through their fans in a unit of time, the amount of food they were able to strain was reduced accordingly. It seemed, therefore, more plausible that the food factor, rather than the dissolved oxygen, would require 3 or 4 days to become effective.

(3) The minimum current rate at which the transplanted larvae became established was found to be 0.58 foot per second in Maple River and 1.26 feet per second in Carp Creek. It is very significant that the minimum rates differed so much in the two streams, one being a little over twice the other. The disparity might be due to either a difference in species or a difference in the nature of the stream.

The material used in Maple River was practically a pure *S. vittatum* Zett. population; that in Carp Creek, a mixture of *S. vittatum* Zett. and *S. venustum* Say. These two were the most common species in the Douglas Lake region and occurred together very often in both swift and moderate currents. They were also found together in other parts of Maple River. It is evident, therefore, that, although the larvae were not identical in the two transplantations, this difference in the current requirement was not one of species.

Now, if the two streams are compared, they are very different in their source of water supply. Maple River is formed exclusively

from surface lake water, while Carp Creek is fed wholly by underground water. The former has a temperature of 19° to 26° C. during the summer months; the latter, from 10° to 14° C. Considering the temperature factor alone, it would seem that the larvae in Maple River, because of a higher rate of metabolism, would require a swifter current than those in Carp Creek to get adequate oxygen and food supply. Yet the result of the experiments was just the opposite, so that other factors should be examined. Both streams have high content of dissolved oxygen, Maple River being about 62 per cent of saturation and Carp Creek about 60 per cent. This shows that it was not because of lower oxygen supply that the larvae in Carp Creek required twice as strong a current as those in Maple River. The other possible factor to be discussed is the food material in the streams. Maple River has a typical lake plankton population, while Carp Creek, originating as it does from springs, has little chance to develop a plankton. Quantitative plankton examinations revealed this fact. If the amount of food that can be strained from a unit volume of water is much less in Carp Creek than in Maple River, it is evident that the larvae in the former need a much larger volume of water to pass through their fans in order to obtain the same amount of food. It seems possible, therefore, to conclude that striking lack of agreement in the minimum rates for the establishment of transplanted larvae is due to the difference in the amount of food material available in the two streams.

Trough experiments in Carp Creek

In the transplantation work two possible imperfections had appeared: (1) the current rate at the different stations, though in general quite uniform, was not perfectly so, and (2) transfer of larvae from their normal habitat to a different one might be too sudden for them to adapt themselves, especially when the new locality had a much weaker current. It seemed advisable, therefore, to contrive an arrangement in which the current rate could be regulated and the velocity be decreased gradually, all other factors remaining unmodified.

Equipment. — The overflow from one end of the beaver dam

came down to a shelf and forward over a natural slope into the creek. This slope, with its abrupt drop of approximately one foot, was utilized for the trough experiments. A water reservoir was built near the edge of the shelf and water was admitted into a parallel series of four long troughs through four openings in the headpiece, which was set firmly at the edge of the slope. Each opening, which was $2\frac{1}{2}$ inches in diameter, was fitted with a movable metal shutter by means of which the amount of water coming through could be regulated. The reservoir above the headpiece provided a steady stream into the troughs, even though the volume of water from the beaver dam might vary from time to time. A side channel served as an outlet or overflow when the water-level in the reservoir came to within 2-3 inches of the top of the headpiece. Each trough was 12 feet long and had a maximum depth of 4 inches. One end rested on a support built on the headpiece just below the opening. The other end was adjustable to various heights, being suspended by wire to a crossbar. With the angle of slope adjustable and the volume of water regulated by the shutters, it was possible to obtain various current rates in the different troughs. Here, then, though all other factors remained the same as in the normal habitat, study was made of one single factor, namely, that of current variation.

Sticks bearing a good supply of larvae were collected from Carp Creek in the immediate vicinity of the troughs and gently introduced into them. They were held in place in the trough with wire. Examinations were made every second day; the current rate was measured and the number of larvae recorded. In most cases an actual count was made. These troughs were set up on June 29, 1927, and were kept under observation until August 16.

RESULTS

Trough 1. — Larvae introduced on June 29 were kept under observation until August 16. They were attached to a stick approximately 4 feet long and slightly more than an inch in diameter. It was comparatively straight, but had two short bends which produced local current accelerations by being too close to one side of the trough. Other local ripples were produced

at the lower end of the stick where there was a drop in the water-level, especially when the amount of water in the trough was cut down. These three points, therefore, always registered higher current rates than along the straight, smooth sections of the stick. This made possible the observation of the larvae at points with small differences in the velocity rates. For the first three days the velocity was about 3.5 feet per second and the larvae were well established. When the velocity was gradually reduced, the results were as follows: (1) During reduction of rate to 1.2 feet per second, the larvae were not affected and did not shift around; (2) When the velocity was cut down to 1.1 feet per second, a few large larvae migrated, but most of them remained; (3) With a rate of 0.9 foot per second, larvae disappeared from some points and remained at others — in general, few *large* larvae stayed in this velocity and then never more than 2 or 3 days, but *medium* and *small* ones persisted at this rate; (4) For small larvae, the rate of 0.8 foot per second appeared to be the critical point, because only twice were a few individuals found in such a slow current; (5) At points with rates lower than 0.8 foot per second, larvae never remained.

Trough 2. — This trough was used for a study of maximum rate. One stick bearing larvae was placed in the trough on June 29 and kept under observation until August 16. The initial current rate was 3.5 feet per second on the first day, but after a gradual increase reached 6 feet per second on the eighth and was maintained thus throughout the rest of the period. This was the highest velocity in this trough set-up. The first count made on the fifth day showed 28 larvae. This number *gradually and slowly* decreased, but there were *new pupae* appearing throughout the whole period. After 43 days there were 3 larvae and 4 pupae on the stick. This record shows that the larvae lived in a current of 6 feet per second for a long period. This rate, however, was not high enough to dislodge them from their attachment. It would have been interesting to determine the exact maximum rate, but this experiment was not extended further because the *minimum rate* was, after all, the critical matter in this work.

Trough 3. — This trough was operated to determine the mini-

imum rate. The results agreed with those from Trough 1. The only additional finding was the record of a mixed group of medium and small larvae established at a point where the rate was 0.8 foot per second.

Trough 4. — For the first 10 days this trough was operated with a current rate of 3.5 feet per second and larvae were well established. It was then changed into a study on slow current. In general, the results were like those from Trough 1.

A similar arrangement of troughs had been set up below the beaver dam in the previous summer (1926), but observations were made over shorter periods and the results were not then considered conclusive. However, when these are compared with the results of 1927, it is significant to find that the minimum rates determined are the same.

Larvae in standing water

It is repeatedly stated in the literature that larvae can live only a few hours in standing water; the longest period recorded is 48 hours (Pomeroy, 1926, p. 19) in shallow water barely covering them. The writer has kept them for much longer periods. The results of these experiments are to be presented and discussed in the section on oxygen supply, but the fact that many larvae have been maintained for 8–14 days and a few for 23 days in standing water should be recorded here. It definitely shows that larvae can live for several days in shallow water *without* any current at all.

EXPERIMENTS ON THE FOOD FACTOR

Filtration of lake water

Equipment. — In order to remove the food material from the water without otherwise changing it, a sand filter was devised. Since the natural medium for *S. vittatum* at the origin of Maple River is formed from surface lake water, the same kind of water was used in the experiment. Current was provided by leading the filtered water into a trough, on the walls of which the larvae were induced to attach themselves.

The filter tank was of galvanized iron, 14 inches in diameter and 28 inches deep. Two drainage openings at the bottom were

fitted with stopcocks to regulate the flow. The gravel foundation consisted of several layers of different sizes, which were as follows, beginning at the bottom: (1) gravels, 1-3 inches in diameter and about 4 inches deep; (2) gravels, $\frac{1}{2}$ 1 inch in diameter and about 2 inches deep; and (3) smaller gravels about 2 inches deep. Superimposed was the sand layer, about 12 inches deep. Tyler graded screens were used to sift the sand, in order to get grains of different sizes in the proper proportions for a filter bed. The sand was taken from the clean, exposed beach and thoroughly washed before it was put into the filter tank. Water was let into the filter at the top from the big water reservoir in the aquarium building. This reservoir was kept filled with fresh, unmodified lake water by a power pump. A wooden trough, 54 inches long, received a steady stream from the bottom drainage outlet of the filter tank. A similar trough was set up as the control, receiving water directly from the main reservoir.

S. vittatum larvae were collected from Maple River on July 18, 1927. A steady stream was first started in each trough, after which larvae were introduced one by one with a pair of forceps. With the larva held loosely between the curved points, the forceps were placed in water against the side of the trough. If the larva was in such a position as to be able to reach the board with its mouth-parts, it clung there and drew its posterior end down against the side to effect attachment. If the larva was washed away from between the prongs, it usually spun a silk thread as it was carried downstream, one end of which remained anchored to the forceps. By gently pressing the forceps against the side of the trough, the silk strand would usually adhere to it, thus bringing the larva also nearer the side to which it might attach itself, either directly or by climbing back on the silk thread until it reached the board. Of course, some larvae were carried away by the stream, particularly if when put into water they did not produce any viscous material on the forceps and therefore could not begin spinning the silk thread. By careful manipulation larvae were introduced without much loss. In order to secure in the two troughs material strictly comparable as regards ages, care was taken to introduce a few of the same size into the experimental

trough, and then the same number of similar size into the control. By repeating this alternate selection process many times, ultimately both troughs were provided with larvae of medium sizes, the proportions of each size thus being as nearly the same as possible in the two troughs. By regulating the amount of water supply and the angle of inclination, these two troughs were kept with approximately the same current rate, 1.4 feet per second, throughout the period of observation. Examination was made twice a day and the number of larvae present, of pupae transformed, of emergences and deaths was recorded.

In order to test the efficiency of filtration by this filter bed, water from the drainage outlet at the bottom was passed through a plankton net, but no plankton organisms were recovered. It was then passed through a fine filter paper, but nothing was collected after 10 or 15 minutes' filtration. It was only after longer periods that a few extremely minute algae might be seen on the paper under the microscope. This study shows that the possible food supply was almost wholly removed by this filtration. When the feeding method of the larvae is considered, the small number of minute algae present became really negligible as food. From the eighth day, the drainage gradually became slower; on the next morning, about one-half inch of surface sand was scraped off. The removal of this coating showed the clean sand underneath and normal filtration was restored. Fresh, clean, graded sand was added to maintain the depth of the sand layer. Only one more scraping was needed to keep it working until the end of the experiment.

Results. — The results from this set of troughs are summarized in Table I. No marked effect was produced on the larvae by keeping them in filtered water. It should be noted that the filtration did not remove every possible food material contained in the water; it merely removed the plankton organisms that normally constitute the food for the larvae. Whether the larvae were able to secure nutrition in the filtered water in some form other than plankton was not determined. It is evident that with a greatly reduced food supply, the larvae still lived to pupation, although it was somewhat delayed. While the last larva in the

experimental trough pupated only 5 days later than that in the control, marked differences were found earlier in the experiment. As shown in the table, 43 per cent of the pupation in the control trough occurred within the first 4 days and 50.5 per cent within the next 4 days, making a total of 93.5 per cent; but in the experimental trough only 39.2 and 24 per cent occurred in the same periods, respectively.

With reduced food, growth should be affected and the mature larvae in the experimental trough should be proportionately smaller than those in the control. Since actual measurements of the larvae could not be taken without removing them from the

TABLE I
EFFECTS OF FILTERED LAKE WATER ON LARVAE AND PUPAE

	Experimental trough Filtered lake water	Control trough Unmodified lake water
Duration of experiment.....	32 days*	26 days
Larvae introduced.....	212 "	220 "
Larvae dead.....	23 "	19 "
Larvae lost.....	59† "	15 "
Larvae pupated.....	130 "	186 "

Duration of Pupation	Number	Percentage	Number	Percentage
Within first 4 days.....	51	39.2	80	43.0
From 5th to 8th day inclusive.	32	24.6	94	50.5
From 9th to 12th day inclusive	39	30.0	10	5.3
From 12th to 16th day inclusive	7	5.3	1	0.5
On 21st day.....	..		1	0.5
On 26th day.....	1	0.7	..	
Total.....	130	99.8	186	99.8

* The last pupa had not emerged when the experiment was discontinued.

† The large number of losses was due to external disturbances; the trough was without a stream for a short period, once on the third morning and another time on the thirteenth day. Except for these losses, the number of larvae disappearing from the trough was comparable with those in the control trough.

trough, such a study was not made, but all the pupal cases in each trough were preserved after the emergence of the adults. Owing to the membranous folds of the abdomen, the pupal skin could not be accurately measured. The cocoon, however, was of firm texture and provided a good index of the size of the larva. *S. vittatum*, *S. venustum* and *S. jenningsi* all have cocoons of the wall-pocket type, with cases of distinctly different sizes corresponding to the size of the larvae of each species. Furthermore, as pointed out in the description of cocoon-spinning, the dimensions of the cocoon were mainly determined by the length of the larva, because it was attached at its posterior end throughout the *whole* process. Therefore the cocoons from these two troughs were measured to get the relative size of the pupating larvae. Measurement was taken from the apex of the cocoon to the middle of the rim at the open end. No attempt was made to get the length between the apex and the two corners in front, because they were not well defined and parts might be broken off when removed from the trough. If the apex of any cocoon was not complete, it was discarded and no measurement taken. Altogether, 167 cocoons from the control trough and 104 from the experimental trough were measured. Table II gives the measurements. The cocoons from the experimental trough were grouped into two sets: (1) those resulting from pupae which transformed within the first 4 days, and (2) those belonging to pupae that transformed after the 4th day. Cocoons from the control trough were also measured in separate lots, but they were put together because there was no difference in size between the early and the later cocoons. The average length is 3.62 mm. for cocoons from the control trough and 3.08 mm. for those from the experimental trough. When plotted, each curve has a mode at a different point: cocoons from the control trough had the highest figure, 3.42 mm.; those from pupae of the first 4 days in the experimental trough the next lower, 3.15 mm.; and those after the 4th day the lowest, 2.88 mm. These figures clearly show the smaller size of larvae in the experimental trough, apparently because of the effect of the much reduced food supply. This experiment shows that larvae of the *medium to mature stages* continued to develop and transform to adults when

the food supply, i.e. the plankton in the water, was almost, if not completely, removed.

Larvae in well water

Standing-water cultures. — In some standing-water cultures, aerated well water was used. This was underground water that had passed through deep layers of sand and was obtained by means of a hand pump and a long iron pipe driven deep into the ground. Microscopic examinations had shown the water to be free of organisms. In starting a culture, although small amounts of lake water and possibly some organisms were introduced adhering to the larvae, the amount of food material that might be present in such a culture was extremely limited. But larvae lived 16 days

TABLE II

MEASUREMENTS OF COCOONS FROM THE EXPERIMENTAL TROUGH WITH
FILTERED WATER AND FROM THE CONTROL TROUGH

Length of cocoon in mm.	Control trough: number of cocoons	Experimental trough: number of cocoons	
		Pupated within first 4 days	Pupated after 4th day
2.34	..	1	..
2.47	..	1	2
2.61	..	..	4
2.74	3	2	14
2.88	12	8	18
3.01	15	6	9
3.15	26	9	8
3.28	24	7	..
3.42	28	6	1
3.55	27	2	2
3.69	14	2	1
3.82	14	1	..
3.96	2	..	..
4.09	1	..	..
4.23	..	..	..
4.36	..	..	..
4.50	1	..	..
Totals...	167	45	59

in one culture; in another, 19 larvae (18.8 per cent of the original population) of various sizes were still alive after having been in well water for 10 days. In these cultures the last pupation was recorded on the 7th or 8th day, and no growth was apparent in the younger larvae.

Well-water trough. — An attempt was made to study the larvae when kept in a stream of aerated well water. Two carboys were used as the reservoir; a siphon was arranged to lead a small, steady stream into a 2-foot trough, on the wooden board of which the larvae were induced to attach themselves. Few were lost from the trough while the stream was running. Under these conditions larvae lived as long as 8-9 days. Since only fresh, pure well water surrounded the larvae, this record can be taken as representing complete starvation.

Larvae in damp chamber

Frequently larvae were left in collecting jars after the contents were poured out. If the jars were stopped tightly and the larvae remained wet some were found alive after 3 or 4 days; sometimes they lived for 7 days. Such larvae had no chance to take food.

EXPERIMENTS ON OXYGEN SUPPLY

Standing-water cultures in open vessels

Cultures. — Previous investigators have claimed that the larvae die within a short time in standing water. They have explained this phenomenon as due to insufficient oxygen supply, but no experiments were performed to support or disprove this supposition. Attempts were made, therefore, to keep larvae in standing water and to study the conditions critically.

Six different sets of experiments were made, all essentially of the same general character. In the first five, 692 larvae of *S. vittatum* were used; in the sixth, about 400 larvae of *S. vittatum* and *S. venustum* mixed. All cultures were kept in the aquarium building, where temperature conditions approximated closely those of the habitats from which the experimental larvae were secured.

Details of experiments cannot be presented here; they are on file in the office of the Graduate School and in the Department of Zoölogy, University of Michigan.

The results show that some of the larvae can live in quiet shallow water for several days. The longest period observed was 23 days for a few small larvae and 14 days for large ones. Even with shorter periods of 6–10 days, during which the majority gradually died, the period was too long to account for the death of the larvae simply on the basis of a lack of adequate oxygen supply in the medium. In a critical examination of the conditions, the following data should be considered.

(1) *The dissolved oxygen content of shallow water in the open vessels.* — Since the quantity of water in these cultures was not sufficient to make a gas analysis, a study was made of the rate of absorption of oxygen by water with very low oxygen content. It was found that in water $2\frac{1}{2}$ inches deep, the oxygen content reached 25 per cent of saturation in 1 hour and 33 per cent in 3 hours. In water 1 inch deep, the oxygen content reached 56 per cent of saturation in $3\frac{1}{2}$ hours and 60 per cent in $4\frac{1}{2}$ hours. After this concentration was reached, it stayed around 60–65 per cent in water $\frac{1}{2}$ – $1\frac{1}{2}$ inches deep, whether the water had stood for one day or several days. This percentage was about the same as that in Maple River and Carp Creek. It is evident, therefore, that the actual amount of dissolved oxygen in the standing-water cultures was not lower than that in the natural habitat and, so far as oxygen content is concerned, ought to be sufficient to meet the requirements of the larvae.

(2) *Temperature and the resistance of larvae in standing water.* — It was observed that collections made on warm days usually had a higher percentage of deaths than those made on cooler days. Furthermore, material from a cold stream, such as Carp Creek, suffered much more in the transportation from the field to the laboratory than did the material from warmer waters. The experiments showed that larvae can endure cold, *standing* water much better than standing water whose temperature is higher than their natural habitat.

(3) *Overcrowding.* — Overcrowding of larvae in the limited

space may be one of the reasons why the majority usually died off within the first day. After the removal of the dead subsequent to this initial loss, the majority of the survivors lived in these cultures for 3-4 days; a few individuals existed for 6-14 days. In the field, the author commonly selected sticks or plant leaves with a large population of larvae and then subjected them to the conditions of a small collecting jar. On being brought to the laboratory, the larvae were seldom divided into different vessels. Since the thick colonies in the natural habitat had fresh water flowing over them, they met a different condition when so crowded and in water not moving. But with reduced numbers they fared much better in the same vessel.

Standing water with known oxygen content

Method. — In order to study the larvae in water with constant dissolved oxygen content, particularly with small amounts of O_2 , a series of experiments was performed. Two 250-c. c. bottles were filled with the same kind of water according to the usual procedure of collecting samples for gas analysis. Into one of them were introduced 5 larvae, after which it was stoppered tightly; the other was used for the dissolved oxygen analysis. In this series of experiments Ann Arbor city water was used. Preliminary experiments had shown that larvae lived well in this water if aerated. It is underground water obtained from artesian wells; analysis had shown it to be practically devoid of dissolved oxygen. When water with very low oxygen content was desired, the tap water was drawn with a rubber tubing inserted into the faucet with the outlet end of the tube kept under the surface of water in a large circular aquarium which was used as the common supply for the two bottles in each set of experiments. To obtain water with a little higher oxygen content, the tap water was allowed to fall gently without agitation on the wall of the aquarium; after various periods of standing, the bottles were filled. For experiments with high oxygen content the water in the aquarium was aerated with compressed air before it was siphoned into the bottles.

A sample for dissolved oxygen analysis was taken at the time when the bottle was filled and another one at the end of the ex-

periment from the water in the bottle. Temperature varied from 14.4° C. to 22.2° C.

The material used in these experiments, *S. jenningsi* Mall. mixed with some *S. venustum*, was collected in the Huron River.

Results. — Details of the experiments will be omitted. The results may be summed up as follows: (1) When the water had a dissolved oxygen content of 15-23 per cent saturation, larvae lived from a few minutes to 13 hours; (2) When the dissolved oxygen content was lower than 15 per cent of saturation, larvae succumbed within a few minutes; (3) In water with a dissolved oxygen content of 72-80 per cent of saturation, the larvae lived 60-114 hours.

Current and low dissolved oxygen content

After it was found that larvae could survive for several hours in standing water with a dissolved oxygen content as low as 15 per cent of saturation, an attempt was made to determine the results when the larvae were supplied with a current, the dissolved oxygen content of the water remaining low. This required an arrangement by which the water had a steady current, but was not exposed to the air. The two kinds of equipment which were used will be described separately.

The Stirrer

Equipment. — A cylindrical aquarium of 10-inch diameter was fitted with an axle which could be revolved freely when its upper end was attached to a motor. Near the lower end of the axle was fixed a transverse bar bearing a metal blade, 2½ inches by 2½ inches, on each end. When the axle was revolved, the blades pushed the water in front of them and thus created a steady current flowing over the wall of the aquarium. This axle was so fitted that it could be easily removed or set up again.

First, the larvae were put into the aquarium, in about 4 inches of water. This was to prevent them from becoming established on the aquarium wall higher than the level of the upper edge of the metal blades. In order to get them attached on the aquarium wall, use was made of the compressed air. In the stirrer

aquarium, when two or three rubber tubings were so arranged as to let the air bubbles come up along the wall, the larvae soon became established near the outlets. When enough were attached for the experiment, all the rest were removed and the axle was set in place. In order to reduce the high oxygen content resulting from the aëration with the compressed air, more tap water was quietly siphoned into the aquarium beneath the water surface. Then a thick layer of paraffin oil was poured over the surface of the water to protect it from the atmosphere. When connected with a motor and set revolving, the stirrer produced and maintained a steady current flowing over the larvae. A similar stirrer was set up as the control, but without the oil layer. In this work the motor was mounted on a power table equipped with a series of pulleys so arranged as to provide a wide variety of different rates of revolution. The axles of the two stirrers were separately coupled up by belts with pulleys giving the same speeds. With the rate of 50 revolutions per minute, the current rate was calculated to be 2 feet per second.

It was soon discovered that the layer of paraffin oil failed to prevent absorption of atmospheric oxygen by the water underneath it, although the rate of absorption was *distinctly retarded*. This agrees with the unpublished work of Professor Paul S. Welch on the efficiency of the oil layer as a means of *preventing* oxygen absorption. While this manuscript was being prepared, Hill (1928, p. 374) reported on the relative efficiency of different liquids for the exclusion of oxygen, likewise confirming the fact mentioned above. According to Harvey (1928, p. 63), Roberts has conducted, with similar general results, experiments "upon the rate of entry of oxygen into sea water covered with a thin film of oil." Therefore, in order to maintain a reduced dissolved oxygen content, part of the water in the stirrer was siphoned from under the oil and water fresh from the tap was similarly siphoned in to restore the original volume; this was repeated as frequently as conditions demanded. Care was taken not to disturb the larvae or to lower the water-level. This exchange of water was performed without stopping the rotation and few larvae were dislodged. By this partial change of water the dissolved oxygen content was kept

lower than that in the control-stirrer, although it could not be kept constant.

Results. — Table III gives the records of Stirrer Experiment I, in which the dissolved oxygen content was not reduced by the change of water. When the amount in the stirrer approached that in the control, the experiment was discontinued. Both experiment and control showed a certain gradual loss in larvae, but it was very similar in both. Also, since in both experiment and control the oxygen content was relatively high throughout the test, the loss in larvae indicated what might be expected in Stirrer Experiment II, apart from any possible additional effect of reduced oxygen. Table IV gives the records of Stirrer Experiment II. In general, the number of larvae lost from the wall was slightly greater in the experimental stirrer than in the control, but there was not any greater loss at the time of reduced oxygen content than during other intervals. In fact, after the oxygen content was at 1.50 c. c. per liter, the larvae did not become restless and very few were lost from the wall during the five hours when the

TABLE III
STIRRER EXPERIMENT 1

Date	Number of larvae and pupae				Temperature		Dissolved oxygen (c. c. per liter)	
	Experiment		Control		Experiment	Control	Experiment	Control
	Larvae	Pupae	Larvae	Pupae				
May 27								
2 P.M.	43	8	64	3	21.2° C.	20.2° C.	3.63	4.60
May 28								
9 A.M.	37	10	61	4	23° C.	20° C.	4.88	5.73
5 P.M.	34	10	60	4			...	...
May 29								
9 A.M.	30	10	57	6	21.5° C.	20.4° C.	5.79	6.42
4 P.M.	28	10	53	6			...	...
May 30								
10 A.M.	22	10	51	6	21° C.	20° C.	6.14	6.49
7 P.M.	18	10	42	6				..

oxygen content was between 1.56 and 2.91 c.c. per liter. The losses in both experiment and control are very similar to those occurring in Stirrer Experiment I, in which reduced oxygen was not involved. Evidently reduced oxygen had no significant effect.

The Rotation Dish

The stirrer experiments showed that larvae were well established in the current when the dissolved oxygen in the water was

TABLE IV
STIRRER EXPERIMENT 2

Date	Number of larvae and pupae				Temperature		Dissolved oxygen (c. c. per liter)	
	Experiment		Control		Experiment	Control	Experiment	Control
	Larvae	Pupae	Larvae	Pupae				
June 1								
2 P.M.	220	..	268	..	21° C.	19.8° C.	3.02	4.41
8 P.M.	205	5	253	6			...	...
June 2								
10 A.M.	194	12	237	14	21.4° C.	19.3° C.	4.26	5.68
3 P.M.	184	15	230	20			...	...
3:10 P.M. *	...	..	...	..			...	...
5 P.M.	172	18	228	22	21.7° C.		2.91	...
June 3								
10 A.M.	150	22	218	30	22° C.	20.2° C.	3.69	6.34
3 P.M.	...	..	...	..			...	...
5 P.M.	144	24	...	..	22.2° C.		1.56	...
10 P.M.	141	24	210	32	23° C.		2.45	...
June 4								
9 A.M.	132	26	190	39	23° C.	22° C.	3.55	6.43

* Partial change of water to reduce the dissolved oxygen content.

varying between 1.56 and 3.70 c.c. per liter at 22° C., that is, from 17 to 41 per cent of saturation. In order to find the minimum limit of the oxygen content for the larvae, the rotation dish experiments were devised. Since the experiments on standing water with low oxygen content showed that some larvae were able to

live as long as 13 hours in water with dissolved oxygen of 15.23 per cent of saturation, it was decided to study the effects of lower oxygen content not only in a current, but also in standing water. In this way the relation between the actual amount of dissolved oxygen and the current might be discovered.

Equipment. — In order to maintain low oxygen content and at the same time a current in the water, tightly sealed vessels were rotated on a platform, which was then connected to a motor. Tap water, known to be almost devoid of dissolved oxygen, was drawn into a large aquarium by letting the water flow gently against the wall with as little agitation as possible. If a very low oxygen content was desired, water was drawn with a rubber tubing inserted into the tap faucet and the outlet end of the tube kept under the water surface of the receptacle. Water was then siphoned from the aquarium into a bacteria dish 7 inches in diameter. After it had overflowed the dish $1\frac{1}{2}$ –2 times its capacity, a sample for oxygen analysis was taken while the siphon from the aquarium kept the dish filled. Then 5 larvae were placed in the dish and induced to attach themselves near or on its wall. A heavy glass plate cover was slipped on so as to include no air bubbles and was sealed tightly with vaseline. This dish was then set on the rotating platform. A control dish was set up with the same water, with exactly the same method, and placed on a table (not rotated). At the end of each experiment, a sample for oxygen analysis was again taken from the water in the dishes.

After the experiment was started, the larvae were examined about every 10 minutes. But in the beginning, or when the larvae appeared to be in a critical condition, an examination was made every 5 minutes. In those experiments which ran over several hours, examinations were made every 20–30 minutes toward the end of the period. When the control dish was examined, the larvae were continuously watched, usually for periods of 1–2 minutes and sometimes even 3–5 minutes. If they showed no movement, the dish was jarred to stimulate them. When they failed to respond to repeated stimuli a “no response” record was made. In the experimental dish the rotation had to be stopped in order to make an accurate observation. Usually the larvae

showed movement upon the stopping of rotation or when the water came to a standstill after 1-2 minutes. Most frequently, they moved in a characteristic way as if testing the current around them, or they might bend sidewise or wave the anterior part of the body. If the larvae failed to move after 2 minutes, then the rotation platform was pounded in order to jar the dish, and their response or failure to respond was recorded. Concerning the movement of water in the rotation dish, it should be mentioned that the current flowing over the larvae was not continuously of uniform rate. When rotation first started, there was considerable lag of the water as compared with the speed of dish rotation, hence greater current, but it slowly decreased until the motion of the water acquired its nearest approach to the rate of rotation of the dish, after which the slow current thus produced against the walls of the dish was uniform. In this set of experiments the important factor for this study was *not the actual current rate*, but rather the difference in the effects on the larvae of standing water as contrasted with moving water. With the water moving, even though slowly, the medium in the immediate surroundings of the larvae is being changed all the time, and this constitutes the significant difference from the condition in the control dish where the water was standing still. Furthermore, with each examination of the experimental dish, the rotation was stopped and started again, so that this increased the rate of water movement for short intervals.

Results. — The results from this series of experiments are presented in Table V. The most significant point is that the larvae in every rotation dish resisted the low oxygen content better than did larvae in the corresponding control, no matter what percentage of saturation each set presented. In fact, the dissolved oxygen in every control dish, except two, was slightly higher than that in the rotation dish, because the former was always set up after the latter and consequently the water in the supply aquarium absorbed more oxygen during this interval. Since the two dishes of each experiment were set up under identical conditions, except that one had the water moving and the other quiet, the difference in results can be attributed only to the factor of current. When

TABLE V
ROTATION DISH EXPERIMENTS

Experiment	Temperature		Dissolved oxygen (c. c. per liter)		Percentage of saturation		Duration of experiments	Results
	Beginning	End	Beginning	End	Beginning	End		
No. Form								
IV Rotation dish	15.8° C	17.7° C	0.25	2.35 *	2.5	24	3 hrs.	2 no response 2 no response after 15 min. 1 no response after 30 min.
Control	16° C	18° C	0.34	0.76	3.4	7.6	3 hrs.	5 no response
III Rotation dish	15° C	18° C	0.34	0.59	3.3	6.1	1 hr., 45 min.	2 no response 1 no response after 25 min. 1 no response after 35 min. 1 no response after 70 min.
Control	15° C	17.8° C	0.59	0.85	5.8	8.8	1 hr.	5 no response
VI Rotation dish	16° C	18° C	0.76	0.97	7.6	10.1	2 hrs., 20 min.	2 no response after 30 min. 1 no response after 38 min. 1 no response after 60 min. 1 responded till end of experiment
Control	16° C	17.8° C	0.85	0.97	8.5	10.1	2 hrs., 30 min.	2 no response 1 no response after 15 min. 1 no response after 20 min. 1 no response after 30 min.

I Rotation dish	14.5° C	19.5° C	0.78	2.98 *	7.6	32	4 hrs.	1 no response after 55 min. 4 responded after 2½ hrs., before glass cover shifted
Control.	14.5° C		0.78		7.6		38 min.	3 no response 2 no response after 15 min.
V Rotation dish	16.1° C	18.4° C	1.28	1.36	13	14	2 hrs., 30 min.	2 no response after 2 hrs., 15 min. 3 responded till end of experiment
Control.	16.1° C	18° C	1.36	1.53	14	16	2 hrs.	2 no response 1 no response after 12 min. 1 no response after 25 min. 1 no response after 30 min.
VIII Rotation dish	14° C	18° C	1.30	1.48	12	15	12 hrs., 25 min.	5 responded till end of experiment
Control.	14° C	17.7° C	1.39	1.52	13	15	11 hrs.	2 no response 1 no response after 1 hr. 1 no response after 2 hrs., 5 min. 1 no response after 3 hrs., 35 min.
VII Rotation dish	16.8° C	18° C	1.36	1.62	13.9	16.8	7 hrs., 25 min.	5 responded till end of experiment
Control.	16.8° C	17.8° C	1.45	1.53	14.8	16	5 hrs., 30 min.	1 no response 2 no response after 40 min. 2 no response after 1 hr., 15 min.
IX Rotation dish	15.8° C	18.5° C	1.65	3.14	16	33	4 hrs., 45 min.	5 responded after 3½ hrs., before glass cover shifted
Control.	15.6° C	18.4° C	1.65	1.69	16	17	5 hrs., 35 min.	1 no response 3 no response after 55 min. 1 no response after 1 hr., 10 min.

* The glass cover slid to one side, thus exposing a small part of the water to air for the last hour.

the slow rate of oxygen diffusion is considered, it is evident that the sedentary larvae will suffer from lack of oxygen when the amount in the immediate surroundings is either exhausted or reduced to a critical point. This supposition also explains how the moving water eliminates this deficiency by providing for a constantly changing supply of the water over the anal gills and body surface. The uniformity of results from this series of experiments gives strong evidence for this function of the current, at least for water with low oxygen content.

The records also show that, with the increased dissolved oxygen, the larvae continued active longer both in the rotation dish and the control. In Experiment VIII, one larva responded after having been in the standing water (oxygen content of about 15 per cent saturation) for $3\frac{1}{2}$ hours. This corresponds closely with results obtained from the standing water series in bottles. In the rotation dishes, when the dissolved oxygen content was 12-14 per cent of saturation, the larvae were still active after 7- $12\frac{1}{2}$ hours' exposure. This record shows definitely that the oxygen requirement of the larvae is not as high as generally claimed.

GENERAL DISCUSSION

Evidence is obtained from this study on the various factors of a normal running-water habitat that *Simulium* larvae have a *definite requirement for current*, and that the high dissolved oxygen content of the water is *not* the determining factor for their presence or absence, as has heretofore been so frequently stated. This is also contrary to the conclusions of Hubault (1927), as described by Zeimat in *Biological Abstracts*, 2 (1928): 832, for the animals living in swiftly running streams. The surface water in the comparatively slower and quieter portions of a stream was found to have practically the same dissolved oxygen content as that of the swifter and more agitated parts; and it is likewise shown by experiments that the larvae survived an exposure of $12\frac{1}{2}$ hours to a low oxygen content of 13 per cent saturation. Therefore, the occurrence of larvae in only the swiftest parts of a stream cannot be explained by the supposition that their oxygen requirement is so high that the slowly moving water cannot satisfy it. When

the natural habitats are examined, as for instance near the source of Maple River, there is a definite minimum rate beyond which no larvae occur. Eggs were frequently found on plants in the shallows, where the current was very weak or wholly absent, and no larvae became established after hatching. On the other hand, along the margin of the lake near the beginning of the river, where the quiet lake water begins to become converted into running water, scattered colonies of larvae were well established in current rates never lower than 0.58 foot per second; a great majority of the rates were higher. Furthermore, the observations on the behavior of larvae after transplantation to currents near the neighborhood of the minimum rate show how they respond to the decrease in the current and desert the new locality for no other cause. It seems, therefore, safe to conclude that the larvae have an *inherent requirement for current*. Furthermore, there is some evidence that the accumulation of sediments may also be influential, to some extent, in determining the suitability of a certain current.

After material is brought in from the field and left in standing water, large numbers die within a short time, as was observed by previous investigators; but the surviving ones live many more days in the same water after the removal of the dead bodies. If the oxygen content in the water failed to meet the requirement of those that died, it should not be able to sustain the remainder. This seems to indicate that the first large mortality was not because the dissolved oxygen present per unit volume was too low, but probably due to some result of overcrowding of larvae in a comparatively small container.

The food factor has also been studied; it proved to be of secondary importance as compared with the current. Larvae have been observed to endure practically complete starvation for 7-9 days, but those of medium and large sizes continued to develop in the experimental trough with filtered lake water, the food supply of which was almost, if not completely, removed. The only effects were that the pupation period was somewhat prolonged and the pupating larvae, as shown by cocoon size, were proportionately smaller than those in the control trough.

SUMMARY

1. New contributions not easily summarized have been made to the general biology of the different stages of *Simulium* and are presented in Part I of this paper.

2. For the first time an observational and experimental study was made of the running-water habitat of *Simulium*.

3. The current rates in the natural habitats were measured and found to be from 0.56 to 2.75 feet per second, or even higher where larvae were attached in more or less vertical falls.

4. By means of transplantation and current experiments in the natural habitat, the following results were obtained:

(a) The minimum current rate for larvae may be different in different streams. The difference in the minimum rates in Maple River and Carp Creek was found to involve a difference in the food supply of the water; in Maple River, with a rich plankton, the minimum rate was 0.58 foot per second, but in Carp Creek, with its great paucity of plankton organisms, the minimum rate was 1.13 feet per second.

(b) The minimum current rate for the larvae in the same stream may be different at different points within short distances, largely determined, apparently, by the rate of sediment accumulation.

(c) The minimum current rate for the establishment on larvae after transplantation may be different, depending on the abrupt or gradual reduction of current, the rate being lower in the latter case.

(d) Upon transplantation to a current much weaker than that in the original habitat, some of the larvae voluntarily deserted the new locality within a few minutes. The number thus disappearing became larger as current rate approached the minimum or fell below it. Since all the physical and chemical conditions, except that of current, were identical, this behavior seems to be a response to the current rate as such and indicates an *inherent requirement for current*. This conclusion is further supported by the observation that in other experiments on reduced food supply or low dissolved oxygen content, but with normal current, the larva did not desert the point of attachment.

(e) The highest current rate obtained in trough experiments, 6 feet per second, proved to be below the possible maximum.

5. The food factor was studied by the use of filtered lake water in current troughs. Larvae of medium and large sizes continued to develop and transform to adults in this water with its much reduced food supply, the only effects being a somewhat prolonged pupation period and a slightly reduced size of the pupating larvae. A small number of larvae have been found to stand practically complete starvation for 7-9 days.

6. The problem of oxygen supply has been studied in both standing and running water. The following results were obtained:

(a) Larvae have been kept in standing water in open vessels for 14-23 days, with the majority living 6-9 days. This contradicts the claim that standing water can never meet the supposed high oxygen demand of the larvae.

(b) Analysis of the dissolved oxygen in water from both the quiet and the agitated portions of the different *Simulium* streams has shown that this oxygen content is practically the same. This fails to support the general supposition that the larvae occur in the agitated water because of its distinctly higher oxygen content.

(c) Experiments involving the use of water with much reduced quantities of dissolved oxygen showed that in such waters the presence of current enabled the larvae to live for prolonged periods, while in the absence of current the larvae succumbed within short periods. The same result occurred even when the standing water had a much higher dissolved oxygen content.

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